

the body is considerably less than the length of the head, which is one fourth of the total (without caudal). Snout of moderate length, pointed, as long as the postorbital portion of the head; eye of moderate size. A skinny adipose lobe occupies the place of the enlarged axillary scales of the pectoral and ventral fins. Back crossed by fourteen narrow brownish bands; a small deep black spot at the end of the lateral line; each caudal lobe with four oblique blackish bands; each dorsal ray with one or two blackish specks.

One specimen,  $4\frac{1}{2}$  inches long.

LXI.—On the so-called Eyes of *Tridacna* and the Occurrence of *Pseudochlorophyll-corpuscles* in the Vascular System of the *Lamellibranchiata*. By J. BROCK\*.

SINCE the investigations of L. Vaillant *Tridacna* has usually been reckoned among the eye-bearing bivalve Mollusca. As the clam-shells, or at least their smaller species, are among the commonest inhabitants of the Indian coral-reefs, I had sufficient inducement, during my residence in the Indian Archipelago in the year 1886, to undertake a careful investigation of these supposed eyes. But owing to the abundance of the tasks which presented themselves on the spot I succeeded finally only in bringing with me to Europe some well-preserved material which has furnished the sole foundation of the following description.

As is well known, the margins of the mantle of the living species of *Tridacna* are splendidly coloured. The observation of the living animals in their natural position is one of the most charming spectacles which the coral-reefs, rich as they are in beautiful forms and brilliant colours, can present, and the enthusiastic descriptions of travellers (Quoy and Gaimard †, Cuming ‡, Vaillant §) are in this particular not in the least exaggerated, as I can affirm from my own experience.

The so-called eyes have no small share in producing this

\* Translated by W. S. Dallas, F.L.S., from the 'Zeitschrift für wissenschaftliche Zoologie,' Band xvi. pp. 270-287. The original memoir is illustrated with a plate (pl. xxii.).

† 'Voyage de l'Astrolabe,' Zoologie, par Quoy et Gaimard, tome iii. (1835), p. 488.

‡ Reeve, 'Conchologia Iconica,' part xiv., Monograph of *Tridacna*.

§ Ann. des Sc. Nat. sér. 3, tome iv. p. 73 (1865).

beauty. They stand out from the margin of the mantle, which is sometimes ultramarine blue, sometimes emerald-green\*, as an irregular row of differently coloured points, sometimes black, sometimes brown †, so that an impression is produced as if Nature, in order to heighten the brilliant spectacle, had set differently coloured gems in the splendid material of which she forms the margins of the mantle. Even upon a superficial examination we easily see that these differently coloured spots adorn the summits of low, obtusely conical elevations, which Vaillant directly characterizes as "eye-tentacles" ("tentacules oculiformes," *l. c.* p. 83). How far this is correct a closer examination of their structure will show.

The considerable size which, as is well known, these animals attain, and the labour necessary for obtaining them (they have to be chiselled out of the blocks of madrepore ‡), at once placed a limit upon the amount of material brought away. My investigations have therefore been made exclusively upon three specimens; but as these furnished me with several hundred "eyes" for examination, the want of very young and of full-grown examples can alone be regarded as injurious to the completeness of the description. My largest specimen, which measured 18 centim. along the margin of the mantle, was killed in very dilute chromic acid (0.25 per cent.), then treated with gradually stronger alcohol; in the case of a second specimen of the same size the margin of the mantle was separated from the living animal and hardened successively in dilute osmic solution and then in alcohol; with a third small example I contented myself with hardening in alcohol. As will be seen hereafter these different methods of

\* In *Tridacna crocea*, Lam., according to Quoy and Gaimard, ultramarine blue, in *T. elongata*, Lam., green, in *T. squamosa*, Lam., which was observed by me, most frequently also green, but with all shades towards blue very frequent, until the animals were pure blue. Moreover the metallic lustre of the colours is so strong that, as Vaillant correctly remarks (*l. c.* p. 73) only comparison with jewels can give even a tolerably good notion of them. The pigment, according to Vaillant (*l. c.* p. 86) is exclusively seated in the epithelium of the mantle. At any rate the coloration disappears immediately in alcohol without leaving any traces; it must also be remarked that nothing is to be found of a "spangle-layer," such as is so generally diffused in fishes with metallic lustre.

† Black in *T. elongata*, observed by Vaillant (which is confirmed by Möbius, 'Beiträge zur Meeresfauna d. Insel Mauritius u. d. Seychellen, Berlin, 1880, p. 322), yellowish green in *T. crocea* according to Quoy and Gaimard, as also from my personal recollections in *T. squamosa*. Unfortunately I cannot now make any definite statement upon this point, as I omitted making a coloured drawing.

‡ The mode of life of *T. squamosa* appears therefore to agree perfectly with that of *T. crocea*, as described by Quoy and Gaimard (*l. c.* p. 488).

preservation have supplemented each other very advantageously for the investigation.

Now, therefore, we may pass to the consideration of the "eye-tentacles." At the very first glance at the margin of the mantle of a *Tridacna* we observe a series of irregularly formed but generally obtusely conical tubercles or warts, which, at variable distances from the margin, upon the inner or branchial surface of the mantle, form a row nearly parallel with the margin of the mantle, and in my largest example, in which the mantle-margin is about 18 centim. long, I count on each side about fifty of these structures. Their distance from each other and from the margin of the mantle is no less irregular than their size and form. While the distance from the margin usually varies between 2 and 5 millim., we find individual warts much further inwards, even as far as 15 millim. The distance of the warts from each other is equally variable. While we sometimes find groups of six or eight together in a close series, a more irregular arrangement in small groups of two or three placed at variable distances apart is by far the most frequent condition.

The form of the larger elevations is generally that of a low hill, which, however, appears seated upon the surface of the mantle not straight, but obliquely, in such a manner that the apex looks towards the margin of the mantle. In the largest structures of this kind the long diameter (by which I mean that perpendicular to the mantle-margin) is usually somewhat greater than the transverse diameter (parallel to the margin), which it may exceed by about one third; in middle-sized tubercles the two diameters are nearly equal, and in small ones the proportion may be in favour of the transverse diameter. In the largest warts observed by me the diameters in question attained the lengths of 3 and 2 millim. As regards the form of all the warts, of whatever size, it is characteristic that their dorsal surface melts very gently and gradually into that of the inner surface of the mantle, while the ventral surface (that turned towards the mantle-margin) descends abruptly, and, indeed, below the level of the surface of the mantle, each wart being surrounded on its ventral side by a semicircular furrow, which stands in the same relation to it as the fosse of a fortress to the bastion. This fosse is very seldom faintly marked or quite effaced.

Between the series of large warts and the margin of the mantle there is a series of smaller structures of a peculiar kind, which are only just visible with the naked eye. Sometimes, but not frequently, the structures now to be described occur also between the larger warts or even beyond them;

but the great majority of them form a continuous series between the larger warts and the mantle-margin, and show exactly the same irregularity of arrangement as the larger warts themselves. At the first glance the two kinds of structures seem to have little to do with each other; frequently the smaller ones appear as mere scar-like shrinkings-in of the surface of the mantle without any perceptible elevation above the surface. But more advanced structures, in which these scar-like shrinkings already surround a slightly convex eminence, show us the transition towards typically constructed warts. These smaller structures are warts in course of development; the only thing remarkable in the process of development is the circumstance that the semicircular furrow which girdles the wart ventrally first sinks into the surface of the mantle, and only then the wart rises up above the inner mantle-surface from the dorsal declivity of this "fosse." This idea is also fully confirmed by the examination of series of transverse sections.

In general the smaller structures just described are so little remarkable in the vicinity of the mantle-margin, even in chromic-acid preparations, that during life the slight differences of relief caused by them will hardly appear at all. On the other hand, it would appear from Vaillant's description that during life these structures also are the seat of an intense pigmentation, and therefore must catch the eye very readily. With regard to them our author says (*l. c.* p. 83):—"In the periphery of the free margin of the mantle the green coloration forms an uninterrupted border, which is adorned with a series of very regularly arranged black spots; near them, but further inwards, there are large projecting tubercles also marked with a black spot; they are more numerous in the vicinity of the branchial aperture, and represent the eye-tentacles."

While there can be no doubt as to the identity of these latter structures (which, moreover, are described more in detail in another place, *l. c.* p. 135) with the larger warts described by me, the agreement of the younger structures with Vaillant's series of pigment-spots along the mantle-margin is a good deal more uncertain. In favour of this view we may cite the similarity of situation and arrangement, although so regular a position as Vaillant describes and figures (*l. c.* pl. viii. fig. 1) does not agree with my objects, and, further, the circumstance that the larger warts are most certainly pigmented. On this point, unfortunately, my personal recollections leave me completely in the lurch.

In order to ascertain the intimate structure of all these

formations of the margin of the mantle, suitably selected portions of the margin were stained with alum-carmine and then divided in different directions into series of cross sections from 0.0125 to 0.01 millim. in thickness. The result was very surprising. From the scanty statements of Vaillant it might have been anticipated that the histological investigation would reveal a highly organized eye. "Notwithstanding the volume of these organs," says Vaillant (*l. c.* p. 135), "which in large individuals measure not less than 2-3 millim. at their base, I was unable, on account of the thickness and opacity of the tissues, which render dissections very difficult, in definitely finding in them the constituents which have been described in some other Mollusca, and especially in the species of *Pecten*. Near the apex of the tubercle there is a spot of dark pigment, which may be regarded as a choroid; and, further, in successful preparations, when such a wart is examined from the side, we see a convex transparent capsule, which might perhaps be assimilated to a cornea." These exceedingly cautious assertions, however, agree so little with the actual conditions that it is difficult to say what Vaillant really saw. For his excuse it may be indicated that his method of investigation must necessarily have exposed him to the most serious illusions.

Let us first of all examine the larger wart-like elevations which stand at a distance from the margin of the mantle. In these the first section in any direction teaches us the important fact that the warts themselves are not eyes or other special organs of any kind, inasmuch as their structure perfectly agrees with that of the mantle. But in the warts themselves there are in small numbers some very peculiarly constructed organs of microscopic minuteness, which perhaps might be eyes and which must here be somewhat closely examined in the first place.

The organs in question have the general form of a shallow flask with a broad belly and a short wide neck. They lie immediately beneath the epithelium and are so oriented that the belly is turned inwards and the neck outwards, so that their long axis is perpendicular to the epithelial surface. Even on the largest warts we do not find more than ten or twelve such organs; on smaller ones fewer in proportion to their size. With rare exceptions, in which we find individual organs on the extreme periphery of a wart, they throughout prefer the median regions; their favourite place is the surface of the wart turned towards the mantle-margin, which descends abruptly towards the crescentiform furrow. Here we find about 75 per cent. of their whole number, the remainder

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being pretty uniformly distributed over the most elevated part of the wart. Exceptionally a flask-shaped organ occurs on the outer declivity of the crescentiform furrow, therefore really outside the domain of the wart itself. The "flask-shaped organs," as we will name them for the present, usually stand in groups of two or three close together, but not unfrequently singly. Their size varies little. I found the greatest long diameter of a well-developed organ to be 0.2 millim., while the greatest transverse diameter amounted to 0.15 millim. This would be sufficient to enable them to be recognized under the lens even in a fresh torn preparation, that is to say, supposing them to be sufficiently differentiated from the surrounding tissue, which I greatly doubt.

The minute structure of a flask-shaped organ is comparatively simple. The whole is surrounded by a thin membrane, visible in sections as a strong contour, and which here and there contains imbedded fusiform nuclei. The chief contents consist of large cells, which in life are probably nearly round, but in my preparations irregularly polygonal, in consequence of the shrivelling, which cannot quite be avoided. These cells also possess a distinct membrane, recognizable as a thick contour; their protoplasm is quite free from granular inclusions, and in life probably perfectly transparent and strongly refractive. In my chromic-acid and osmium preparations it had acquired a finely reticular character, no doubt a phenomenon of coagulation; while in the alcoholic preparations a dully-lustrous fat-like substance had separated in large drops. The remarkably small, perfectly spherical nucleus is placed quite excentrically on a part of the membrane. The greatest diameter of these "transparent cells" is 15-25  $\mu$ , that of their nuclei 3-5  $\mu$ .

These transparent cells are surrounded, like a nut by its shell, by a somewhat differently constituted layer, which extends directly inwards from the external limiting membrane. This "external layer" is most perfectly developed at the bottom of the bellied part of the flask-shaped organ (which lies away from the surface of the mantle), and thence advances forwards, but without ever attaining the foremost part, that is to say, to keep up the comparison, the mouth of the flask. On the whole, this cell-layer is characterized by great irregularity. Not only do the individual cell-elements composing it often project irregularly into the cavity of the flask, but their arrangement is also sometimes interrupted; there occur in it larger and smaller gaps, into which the transparent cells penetrate and thus come into direct contact with the limiting membrane. In contrast to the

transparent cells those of the outer layer appear very opaque, owing to their coarsely granular protoplasm; they are somewhat smaller (10–15  $\mu$ ) than the transparent cells, show no recognizable membrane, and are polygonally pressed against one another. Their round nucleus, averaging 3–5  $\mu$  in diameter, is not placed excentrically, but more in the centre.

It is exceedingly remarkable and ought to be particularly noted that I have never seen a nerve passing to a flask-shaped organ.

With the anterior neck-like portion the organs frequently reach immediately under the epithelium, but just as often the thin layer of connective tissue, which forms the boundary between the tissue of the mantle and the epithelium, intervenes between them. The epithelium is the moderately thick, one-layered, vibratile cylinder-epithelium, with basal nuclei and thin, strongly refractive cuticle, which is sufficiently well known in the mantle of the Lamellibranchs. On the outer surface of the mantle (the shell-side) a great number of elongate, flask-shaped, unicellular mucus-glands open among the epithelium; these, when stained with alu-mcarmine, show very distinctly the framework-substance recently described by List \* and myself in these elements.

The flask-shaped organs often project outwards more or less strongly, so as to push out the epithelium covering them in a conical form. This peculiarity is generally much better developed in those organs which we find upon the smaller (and probably younger) warts than in those which belong to older structures. With regard to their epithelial coat also a distinction may be set up. Throughout this is somewhat thinned over the flask-shaped organs, but in the younger much more than in the older ones. While in the latter the difference from the normal epithelium is but small and often scarcely perceptible, the epithelial covering over the younger structures is often reduced to a pavement-epithelium scarcely visible in profile.

We have just spoken of older and younger warts, and this gives the opportunity of going somewhat more into detail upon the relations of the two structures, which in description we have in the first place treated separately. If we examine the minute structure of one of the undeveloped structures near the margin of the mantle, which appear to the naked eye rather as scar-like indrawn depressions, it is soon seen that no principal differences from the larger warts exist in

\* J. H. List, "Zur Kenntniss der Drüsen im Fusse von *Tethys fimbriata*, L.," Zeitschr. f. wiss. Zool. Bd. xlv. p. 281; and J. Brock, *ibid.* xlv. p. 333.

them. Here also we find flask-shaped organs in the typical position at the inner wall of the "fosse," or more rarely upon the still very low dome of the wart which is just swelling up. But their number is small, varying only from one to three; and moreover, as already stated, they project more strongly above the surface, and push out the much thinned epithelium of the mantle into a bump at this spot.

With respect to size and the details of the minute structure there is, however, absolutely no difference between them and the flask-shaped organs of the larger warts. If we examine more exactly the external relief of these younger structures it is not difficult to find evident incipient stages, in which only a deep narrow inversion of the epithelium of the mantle represents the first trace of the future "fosse," while within this the future tubercle is either not indicated at all or only by a broad, low, scarcely perceptible elevation. From these incipient stages up to typically developed warts all possible intermediate forms may be found, and hence there seems to us to be no doubt that the smaller structures near the margin of the mantle are developmental stages of the typical large warts. If this conclusion be correct, this development has certainly the remarkable peculiarity of showing that first of all the "fosse" surrounding the wart on the side towards the mantle-margin sinks in, and the wart only then begins to swell up above the surface of the mantle. If we add to this that our younger specimen of *Tridacna*, in which the mantle-margin measures only 13 centim. in length, shows only such younger organs towards the margin, and, indeed, in rather small numbers (in all about fifty were counted upon each mantle-margin), and, on the other hand, not a single distinctly projecting wart, we are not unjustified in coming to the conclusion that during the whole life of the animal new-formation of warts goes on continually, starting from the margin of the mantle\*.

With regard to the formation of the flask-shaped organs we have unfortunately no direct observation. But from the circumstance that they occur typically developed in the youngest warts, it at least follows with some certainty that their formation precedes that of the warts. Therefore as the place where a new flask-shaped organ originates is not marked externally by change in relief of the surface of any kind, it is clear that only some very fortunate chance could throw any light upon the production of a flask-shaped organ in the adult

\* The development of the warts sometimes attains such an extreme degree that they begin to be constricted off from the parent-surface and give origin to short-stalked clavate or mushroom-like structures.



animal. It may, however, be admitted that for a long time a new-formation of such organs must take place in the larger warts, for a comparison of the number of flask-shaped organs of the larger warts with the very much smaller number in younger structures leaves only the alternative between this supposition and the much more improbable one of a subsequent fusion of several smaller warts into one large one; but even here it is remarkable that I have never been able to observe a flask-shaped organ *in statu nascendi*. However, I will readily admit that I have not devoted much time to seeking for it, as in connexion with the chief interest which attaches to these mysterious organs, namely their function, no light is to be expected from developmental history.

Throughout, in all attempts to attribute any definite function to these organs, we find ourselves in a peculiarly unfavourable position. In the first place, because there are no available observations as to the behaviour of the living animal, as to undoubted sensorial perceptions, or the like, which might be brought into connexion with the flask-shaped organs. Further, because we know nothing definite as to the pigment, which, as we have seen, is undoubtedly present during life, and its arrangement relatively to the organs, a point which would have to be considered in the first line in every attempt at interpretation. And, finally, because we know even the histological elements which compose the flask-shaped organs only in the preserved state. We do not know whether the transparent cells are as strongly refractive during life as we suppose them to be from our preparations; we know nothing as to the constitution of the cells of the "external layer" during life. Thus any serious attempt at interpretation must for the present remain in suspense. Nevertheless we believe we shall not go wrong in decidedly rejecting any interpretation of our organs as eyes. The only things that might be adduced in favour of this interpretation is the resemblance of the "transparent cells" to the lenses of many Invertebrates, and above all the exceedingly favourable position of the organs for visual perception; but how many and important are the reasons against it! Above all, at any rate, the want of any large nerve-trunk running to the organ and of a perceptive layer, for we cannot expect even the boldest imagination to regard the "external layer" as a retina\*.

\* We know very well that precisely among the Mollusca "eyes" have frequently been described to which no large nerve-trunks could be proved to run; but in all these cases the interpretation, although assailable, is to a certain extent justified, if only because the organs in question agreed

A much more probable interpretation is that the flask-shaped organs are luminous organs. If the cells of the "external layer" have the faculty of shining, the "transparent cells" might perhaps act as prisms. This depends, however, very much on whether the distribution of the pigment, which we do not know, supports such an interpretation. Whether any luminosity really occurs during life is not known\*, and, indeed, not at all probable, as from the abundance of the *Tridacna* in the whole Indo-Pacific region so remarkable a phenomenon could hardly have remained unobserved until now.

Perhaps my respected friend Dr. Sluiter, when these lines come under his notice, may be induced to make some observations upon the point in question. With the exception of some superficial and illusory resemblances there is no relation to the luminous organs of the Scopelidæ. The only organs

closely in structure with undoubted eyes (*Patella*, see P. Fraisse, Zeitschr. f. wiss. Zool. Bd. xxxv. p. 468; moreover, as I now find, Hilger has recently demonstrated the nerve of the eye of *Patella*, see Morph. Jahrb. Bd. x. p. 358, 1884), or at least because physiological experiments proved the animal to be extraordinarily sensitive to light (as especially in the cases recently described by Sharp and Patten; see B. Sharp, "On the Visual Organs in Lamellibranchiata," Mitth. Zool. Stat. Neapel, Bd. v. p. 447, and W. Patten, "Eyes of Molluses and Arthropods," *ibid.* Bd. vi. p. 542). But no special sensitiveness to light can be absolutely proved in *Tridacna*; indeed, *Tridacna* is so little sensitive that usually it is only upon direct contact that it retracts the margins of the mantle and closes its shell.

\* I certainly thought that I was on the track of a conclusive observation when I read as follows in the treatise on the Invertebrata, edited by O. Schmidt, in Brehm's 'Thierleben' (ed. 2, Bd. x. p. 387):—"Besides many singular things, as, for example, that the Giant Clams (*Tridacna*) when they open at night diffuse a bright light or a lustre noticeable from a distance . . . besides these things our Dutchman (Rumph) cites some examples of the size and strength of *Tridacna gigas*," &c. But a comparison with the original showed that O. Schmidt had either read the passage in question hastily or misunderstood it. At p. 132 of his 'Amboinsche Rariteitskamer' (first Amsterdam edition of 1705) Rumphius says:—"They relate many singular things of a large *Bia garu* (*Tridacna gigas*) which is to be seen in a lagoon of the island Timor Laut, which on opening at night is said to emit a bright light or lustre, which may even be perceived from afar." Thus it is only a pleasant tale of the natives. It may be remarked *en passant* that Rumphius is well known to have been a German, born in Hanau, as, indeed, is to be read upon the title-page of his 'Rariteitskamer' and also under his portrait behind it, although "Totus Belga fide et calamo," as is added with an elegant compliment to his adopted country in the distichs in his honour placed under the latter. As, in my eyes, Rumph, although a dilettante, was a naturalist of the first rank, who far exceeded most of his contemporary professional naturalists not only in accuracy of observation, but also in critical acuteness, I would not let pass this opportunity of correcting an error which may easily receive the widest diffusion through so popular a work as Brehm's 'Thierleben.'

which show in their structure a decided resemblance to the flask-shaped organs, the so-called "eyes" on the tentacles of *Cardium* (see Patten's figure, *l. c.* Taf. xxxi. fig. 112), are unfortunately very doubtful as regards their function, although the opinion that they are luminous organs is by no means to be regarded as disproved\*.

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It is well known that the symbiosis of unicellular Algae with Evertabrata of the most different classes discovered by Geza Entz and K. Brandt has never ceased to be a matter of the most lively interest. I believe, therefore, that I shall earn the thanks of many by adding to the known cases of this symbiosis a new one which may claim the greater interest as only one instance of the kind among the Mollusca is to be found in literature. The very first incision which I made through the mantle-margin of a *Tridacna* showed me, to my astonishment, all the available interstices of the tissue densely packed with "green cells" (pseudochlorophyll-corpuscles), and, as I found in the course of my investigation, this condition prevailed in all my specimens and in all parts of the mantle. It is true that the interest which would otherwise attach to this discovery was considerably weakened by the circumstance that it was made only on preserved animals. As the strict scientific proof that the colouring-matter of these green cells is chlorophyll can no longer be produced, readers of too critical a disposition may be inclined in regard to the new discovery to pass to the order of the day. In opposition to this I would point out that my only purpose here is to communicate briefly the observations made, which I am justified in doing, and, to a certain extent, obliged to do. When once attention is directed to these things the investigation of the questionable chlorophyll in fresh material will probably not have to be very long waited for.

The "chlorophyll-corpuscles" in question are certainly true cells, as, without exception, they possess an approximately central small nucleus with a distinct nuclear framework, which becomes very deeply coloured in the staining reagents employed (Grenacher's alum-carmine). In general the nucleus is spherical, but sometimes oblong or reniform,

\* Carrière ('Die Sehorgane der Thiere,' Munich, 1885, p. 97) certainly observed no spontaneous luminosity when the outer light was excluded; but a function dependent upon nerve-influence, such as luminosity certainly is, need not occur at all times.

and not unfrequently, especially in alcoholic preparations, strikingly stelliform, to which Prof. Graf Solms called my attention. The increase by transverse division, which is frequently to be observed, may also be cited among the criteria of cell-nature. Further, they are spherical bodies of 6–8  $\mu$  in diameter (nucleus 2  $\mu$ ), the contour of which appears so sharply and definitely that the assumption of a special (cellulose?) envelope\* seems to be justified. From the numerous vacuoles which permeate it the protoplasm has a frothy character; usually a ring of larger vacuoles surrounds the nucleus, and between this and the membrane there are numerous smaller ones. But the most multifarious other arrangements also occur. The green colouring-matter, which is fixed by chromic acid but extracted by alcohol, is not generally diffused through the protoplasm, but localized in small round corpuscles (chlorophyll-bearers), which are distributed through the cell in variable numbers difficult to determine. However, their quantity suffices to cause the whole cell to appear of a lively green colour under low powers. Whether the green granules are situated in the vacuoles or in the protoplasm is difficult to decide from sections; but I regard the latter as far more probable.

Other points in the structure of the pseudochlorophyll-corpuscles I have been unable to make out clearly. In spirit-preparations, in which, as already stated, the green colouring-matter has been entirely extracted, the granules of the protoplasm, which bore the colouring-matter, have also become very indistinct, as their refractive power too nearly approaches that of the rest of the protoplasm. It is only where (in the microscopic image) they lie over a large vacuole that they are very distinctly visible. The vacuoles, however, in their form and distribution are, on the contrary, particularly clearly seen in spirit-preparations from which the colouring-matter has disappeared. The very fine, strongly refractive, almost dust-like granules which I sometimes found scattered through the protoplasm have remained quite inexplicable to me as regards their nature and significance; but I must mention that on treating sections of *Tridacna* hardened in osmium with iodized solution of iodide of potassium for a very different purpose (see p. 450), fine, dust-like, violet-blue granules made their appearance in many of the green cells, while in spirit-preparations chloride of zinc and iodine coloured the whole of the cell-contents deep blue-black. How these two results are to be reconciled, and whether the blue granules are iden-

\* The test with chloride of zinc and iodine was not unequivocally successful.

tical with the above-mentioned granulations, I do not know; but at any rate it may be regarded as certain that the cell-contents contain starch.

The seat of the green cells is not determinable at the first glance. It is indeed quite clear that they never occur intracellularly, like the yellow cells of the Actiniæ for example. For this indeed the tissues of the Mollusca are much too small-celled. But whether they are in the interstices of the tissue or in the blood-passages is more difficult to decide without injections. Fortunately the numerous blood-corpuscles intermixed with them help us into the right road; we have to do only with blood-sinuses, as, moreover, the form and distribution of the spaces filled with the green cells would almost alone render certain. The injection effected by them is frequently so perfect as to give us a distinct picture of the lacunar system in the mantle; and of the subepithelial layer of the mantle-tissue we sometimes obtain representations which to some extent resemble those recently given by P. Schiemenz\*. In this way we are taught that the flask-shaped organs must be surrounded by large blood-sinuses, as an enormous accumulation of green cells regularly occurs around them. On the other hand, in the larger vessels with distinct walls which are distributed in the mantle I have never met with green cells.

The only observation of the occurrence of pseudochlorophyll-corpuscles in Mollusca that literature has to show is due to K. Brandt and relates to *Elysia viridis* †. In this species they lie in the "system of contractile tubes in the mantle;" what Brandt means by this is somewhat obscure, but we may not be much mistaken in assuming that the vascular system is referred to. This would agree with *Tridacna*; but in other respects there is considerable difference in the green cells of *Elysia* according to Brandt's description and figures (*loc. cit.* figs. 90-93). They are much smaller and of much more irregular form than those of *Tridacna*, and the emerald-green colouring-matter is not localized in separate granules, but uniformly permeates the protoplasm.

As is well known Schmitz ‡ has recently adduced evidence

\* Paulus Schiemenz, "Ueber die Wasseraufnahme bei Lamellibranchiaten und Gastropoden," II., Mitth. Zool. Stat. Neapel, Bd. vii. Heft 3, Taf. xvi. figs. 8, 9.

† K. Brandt, "Ueber die morphologische und physiologische Bedeutung des Chlorophylls bei Thieren," in Mitth. Zool. Stat. Neapel, Bd. iv. p. 243.

‡ F. Schmitz, "Die Chromatophoren der Algen," in Verh. naturh. Ver. preuss. Rheinl. und Westf., Jahrg. xl. (1883) p. 1.



that the old supposition that the chlorophyll is uniformly diffused in the protoplasm in the unicellular Algæ is erroneous; on the contrary, according to him all true Algæ have formed chlorophyll-bearers. We cannot abstain from mentioning this memoir at any rate in passing, as our own observations agree so well with it; moreover it is known that in the majority of the green corpuscles found in animals the chlorophyll is localized in special chlorophyll-bearers. The question whether the green cells of *Tridacna* are true unicellular Algæ or only developmental stages of them must be very superfluous when even the vegetable nature of these structures cannot be established with perfect certainty. Upon this point it may be noted here *en passant* that the latter opinion, put forward by Geza Entz, on account of which Brandt gave up his generic name *Zoochlorella*, has recently been disputed by Klebs\* in the most decided manner. And it cannot be denied that of the diagnosis which Klebs (*loc. cit.* p. 332) gives for the genus *Pleurococcus* much is applicable to the green cells of *Tridacna*; but we need hardly say expressly that we will not therefore announce the latter as a new species of *Pleurococcus* †.

The place in which we find the green cells in *Tridacna* is unusual. The ordinary locality of the vegetable symbionts is in the tissue, *i. e.* the cells of the host; hitherto they have only rarely been found floating freely in the cavities of its body. If we interpret Brandt's expression correctly (see p. 447) *Elysia viridis* is also in the same case; in the Ephyrae of *Cotylorhiza*, Claus found chlorophylloid Algæ freely floating in the gastrovascular space ‡; Chun refers to yellow cells in the vessels of *Verella* §; and Silliman saw Algæ deposited in the intercellular spaces of the body-parenchyma in a North-American freshwater Turbellarian, *Mesostoma viviparum*,

\* G. Klebs, "Ueber die Organisation einiger Flagellatengruppen und ihre Beziehungen zu Algen und Infusorien," in *Unters. botan. Inst. Tübingen*, Bd. i. p. 233.

† At any rate, as Prof. Graf Solms has likewise had the goodness to point out to me, the green cells of *Tridacna* are quite different from those of other classes of animals if only by the great number and spherical form of their chlorophyll-bearers. The green cells of *Hydra* have a single hood-like chlorophyll-bearer, and increase by tetrad-formation (see, for example, the figures given by Hamann, *Zeitschr. f. wiss. Zool.* Bd. xxxvii. Taf. xxvi. figs. 4-7), as also those of the Infusoria.

‡ C. Claus, "Die Ephyren von *Cotylorhiza* und *Rhizostoma* und ihre Entwicklung zu achtarmigen Medusen," in *Arb. Zool. Inst. Univ. Wien*, Bd. v.

§ C. Chun, "Ueber die geographische Verbreitung der pelagischlebenden Seethiere," in *Zool. Anz.* 1886, no. 215, p. 72.

Sill.\* I am not acquainted with other cases of the same kind. But, at any rate, the Algal vegetation in the system of blood-lacunæ in the mantle of *Tridacna* proves that there can be no question of any "current" of blood in them worth mentioning. However, it cannot be denied that Algæ (always supposing them to be such) must yield a very valuable enrichment of any animal blood, as the oxygen which they develop under direct exposure to light must be immediately absorbed by the blood-plasma, and so benefit the animal to a great extent. That there can be no question of even a temporary or partial nourishment by the vegetable symbionts in the case of an animal so large and requiring so much nutriment as a *Tridacna* is a matter of course, even if this theory, set up by G. Entz and Brandt, were not to be regarded as already seriously shaken.

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Some minor observations made in the course of the above investigation, but which have no other connexion therewith, may here find a place in conclusion. One of my observations relates to the blood-corpuscles. In my preparations I found these always fixed in a peculiar manner. The protoplasm had distinctly separated into two different constituents—a perfectly hyaline part in which the nucleus was always situated excentrically, and a "protoplasmatic" part, which showed a very marked fibrous coagulation. It was remarkable that in all three of my *Tridacnæ*, which had been treated with quite different reagents, namely chromic acid, alcohol, and osmium, the blood-corpuscles appeared altered in this manner, and, indeed, in each preparation the whole of them without exception, not only those of the deeper layers of tissue, but also those belonging to the subepithelial layers, which one would have supposed must have been killed and fixed almost instantaneously by the reagent employed. I am at present quite unable to offer any explanation of this peculiar phenomenon.

The following peculiarity of the blood of *Tridacna* may have more interest. I believe that the only known formed constituents are the ordinary amœboid blood-cells. But in *Tridacna* I succeeded in detecting, although very sparingly, a second very characteristic cell-element of the blood. These were rounded or oval, lobate, or otherwise irregularly formed

\* Silliman, "Beobachtungen über die Süßwasserturbellarien Nordamerikas," in *Zeitschr. f. wiss. Zool.* Bd. xli. p. 62.

cells, the perfectly hyaline protoplasm of which is throughout so completely stuffed with strongly refractive granules of a fatty lustre that I could not even find a cell-nucleus. The granules of the contents, averaging  $0.5-1 \mu$  in diameter, are irregularly polygonal rather than rounded; in osmium they become brown rather more strongly than the protoplasm, and also acquire a deep colour in borax-carmin.

We find these "granule-cells," which usually attain twice or three times the size of the ordinary blood-cells, intermixed with these and the Algæ in the blood-lacunæ, where they generally lie close to the walls, often in recess-like depressions. If such a depression is seen from the side in a section peculiar images are produced, as though the cell lay free in the interlacunar tissue; and misled by this, I thought for a long time that I had to do with true wandering cells, but I gave up this notion on finding that the explanation above given is quite sufficient.

The frequency of the "granule-cells" is very variable. They are wanting in none of my three individuals of *Tridacna*; but while in the specimen treated with chromic acid and alcohol they always occur singly and so sparingly that I often had to examine several sections in order to find one, they occurred in the osmium-specimen in such abundance that every section showed at least half a dozen of them. The causes of this phenomenon are quite unknown to me.

In order to understand these peculiar cells it is most necessary to make out the chemical nature of their contained corpuscles. For reasons which are not far to seek I at first thought of glycogen; but I did not succeed in obtaining the characteristic glycogen-reaction with a solution of iodine and iodide of potassium prepared in accordance with Barfurth's prescription\*. Whether this failure is to be ascribed to the hardening with osmium or to the processes of imbedding in paraffin may be decided by better chemists than myself—at any rate from the rarity of the "granule-cells" in my other two *Tridacnæ* I was limited to the osmium-specimen for this microchemical test.

The resemblance of the "granule-cells" to certain cells of the interstitial connective substance of the Pulmonata, which were discovered by Semper and reinvestigated and further described by me some years ago †, is very remarkable. The

\* D. Barfurth, "Vergleichend-histochemische Untersuchungen über das Glycogen," in Arch. für mikr. Anat. Bd. xxv. p. 260.

† J. Brock, "Untersuchungen über die interstitiellen Binesubstanzen der Mollusken," in Zeitschr. f. wiss. Zool. Bd. xxxix. p. 40 (1883).

reaction of the contained granules with osmic acid and basic colouring-matters agreed exactly in the two kinds of cells. In the Pulmonata also no evidence of glycogen was adduced, but that we have to do with glycogen or a similar body is at least probable since we know from Barfurth \* that in the Pulmonata glycogen is at times accumulated in great quantity in the plasma-cells or Leydigian cells of the interstitial connective substance.

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The much-discussed question of the intercellular spaces of the epithelium of the Mollusca has now, we believe, found its definitive solution in the recently published memoir by Schiemenz †. The intercellular spaces are not artificially produced, although their supposed stomata may be; they do not reach the surface of the epithelium, but terminate cæcally in sharp points between the epithelial cells. Although the existence of intercellular spaces has been placed beyond a doubt by means of injections (Schiemenz, Nalepa) and by the observation of fresh objects (Leydig &c.), the question deserved consideration how far intercellular spaces may be artificially produced by reagents causing hardening and shrivelling. Schiemenz has already raised this question when he brought forward this very objection to my observations of intercellular spaces in the epithelium of the pedal glands of the Pulmonata ‡. In this particular case Schiemenz's scruples were unfounded, for no one will doubt that intercellular spaces which regularly function as the efferent ducts of glands, and are often found filled with the secretion of the glands, are formed during life §. But as to the matter itself he is undoubtedly in the right. Of my three *Tridacnae* the osmium and chromic-acid specimens showed a densely closed palisade-epithelium without the smallest interstices between the individual cells, while the spirit-specimen has the whole epithelium traversed by numerous large typical intercellular spaces. Only one of the two can represent the natural con-

\* Barfurth, *loc. cit.* pp. 325 *et seqq.*

† Paulus Schiemenz, "Ueber die Wasseraufnahme bei Lamellibranchiaten und Gastropoden," II., in *Mitth. Zool. Stat. Neapel*, Bd. vii. Heft 3.

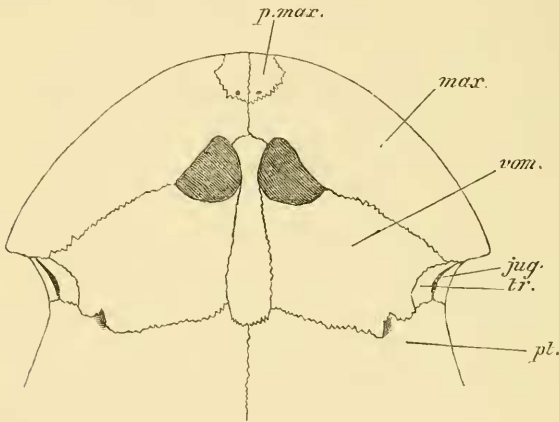
‡ Schiemenz, *loc. cit.* p. 428.

§ Although I have in the same place adduced the intercellular spaces of the epithelium of the pedal glands as evidence for the existence of such formations in general, this can no longer apply now that we know the true nature of the permanently closed typical intercellular passages. The two are quite different things, which must be kept apart.

dition, and from the prevalent opinions as to the value of the three reagents employed we must unanimously declare against the spirit-specimen and the intercellular spaces. Others also appear to have made similar observations. In a recent French memoir upon the histology of the Lamellibranchs\* we find the epithelium everywhere represented as closed, only one figure shows, exactly like my spirit-specimen of *Tridacna*, the epithelium traversed by numerous "intercellular spaces."

LXII.—*On the Presence of Ossa transversa in a Chelonian.*  
By G. A. BOULENGER.

THE object of this note is to record the presence of transverse bones in the skull of *Hydraspis Hilairii*, Schw. The absence of that element had hitherto been regarded as characteristic of the order Chelonia.



Lower view of anterior part of skull.

As may be seen from the above figure, the bone (*tr.*) is intercalated between the pterygoid, the palatine, the maxillary, and the jugal; it is suturally united with the latter only anteriorly and posteriorly, its outer border being free.

\* L. Roule, "Recherches histologiques sur les Mollusques lamellibranches," in *Journ. Anat. et Physiol.* tome xxiii. (1887), p. 31. The figure referred to is pl. v. fig. 8.